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The Impact of Cannabinoids on Breast Cancer Cell Lines: A Meta-Analysis and Systematic Review of Antitumoral Effects

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List of Abbreviations

CBD:Cannabidiol

Δ^9-THC: Δ^9-tetrahydrocannabinol

CCSQEG: Cell Culture Study Quality Evaluation Guide

TAMs: Tumour associated macrophages

IARC: International Agency for Research on Cancer

ER: Oestrogen receptor

PR: Progesterone receptor

CBN: Cannabinol

IC50: 50% inhibitory concentration

HER2: Human epidermal growth factor receptor 2

TNBC: Triple Negative Breast Cancer

CB1: Cannabinoid receptor 1

CB2: Cannabinoid receptor 2

TRPV: Transient receptor potential vanilloid

PPARγ: Peroxisome Proliferator-Activated Receptor-γ
CBG: Cannabigerol
OR: Odds ratio
PI: Propidium iodided
CI: Confidence Interval
ROS: Reactive Oxygen Species
7-AAD: 7-aminoactinomycin D

Abstract

Breast cancer remains a major global health concern with limited treatment options. Metastasis and resistance to conventional therapies contribute to the poor prognosis associated with this disease. In recent years, interest has grown in exploring the potential of photochemical, such as cannabidiol and Δ9-tetrahydrocannabinol derived from *Cannabis sativa*, as alternative anticancer agents. This systematic review and meta-analysis aim to investigate the ant proliferative effects of cannabidiol on breast cancer cell lines, particularly triple-negative and receptor-positive cells. A comprehensive search of electronic databases was conducted to identify relevant studies published in English and Spanish. Eligible studies were assessed for quality using the Cell Culture Study Quality Evaluation Guide (CCSQEG) and data extraction was performed. Nineteen studies were included in the qualitative synthesis, and ten studies were thoroughly analyzed for quantitative assessments. The results demonstrated that cannabidiol exhibited significant ant proliferative effects on breast cancer cells, irrespective of receptor status. The weighted average of the logarithms of odds ratios revealed a strong association between cannabidiol administration and inhibition of tumour growth in breast cancer cells (OR = 0.531, 95% CI: 0.429-0.656, $p < 0.001$). These findings suggest that cannabidiol has the potential to be a therapeutic agent for breast cancer. However, more comprehensive studies are required to elucidate the underlying mechanisms and optimize treatment strategies involving CBD in breast cancer.

Keywords

Breast cancer; Cannabis; Cannabidiol; Proliferation.

Introduction

Cancer is a significant global public health concern and is the second leading cause of death in the United States [1]. As stated by the International Agency for Research on Cancer (IARC), of all cancer types, breast cancer is the most frequently diagnosed cancer worldwide [2]. Despite the progress in early detection methods and therapeutics, breast cancer remains the fifth leading cause of cancer related deaths globally, with approximately 685,000 women affected by this disease in 2020 [3]. Additionally, by 2025, it is estimated that 2.5 million new cases will be diagnosed and the mortality of female breast cancer patients will rise to 769,000 [4-5].

The prognosis of cancer depends on the occurrence of cancer metastasis. During metastasis, several cellular and molecular components play an important role in the dissemination from the primary tumor site to distant parts of the body through mechanisms such as proliferation, migration, invasion, and epithelial to mesenchymal transition (EMT) [5-6]. The capacity of tumors to metastasize is related to tumour phenotype, mutations in tissue-specific stem cells, cell signaling pathways, and the support of immune cells such as tumor-associated macrophages (TAMs) [7]. All these processes allow the tumor cells to avoid the immune system response, alter the tissue microenvironment, and develop resistance to treatments [8].

Resistance to cancer treatments is associated with various pathways that are triggered by specific receptors [9-10]. According to the American Cancer Society, breast cancer is classified into three primary subtypes: estrogen receptor (ER) or progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (HER2) positive with or without ER or PR positivity; and triple-negative breast cancer (TNBC) [11-12]. Clinical evidence suggests that TNBC has a poorer prognosis compared to receptor-positive tumors, owing to its high tumor grade, larger size, distinctive profile, aggressive metastasis and lack of targeted treatment options [11-13].

Several compounds have emerged as potential alternatives to conventional therapies [14]. Researchers have recently turned their attention to phytochemicals present in Cannabis sativa, specifically cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC), as promising antitumor agents [15]. CBD is a prevalent non-psychoactive phytocannabinoid in cannabis extracts that shows strong binding affinity towards a range of receptors, including Type 1 cannabinoid receptor (CB1), Type 2 cannabinoid receptor (CB2), GPR55, transient receptor potential vanilloid (TRPV), and peroxisome proliferators-activated receptor gamma (PPARγ) [16]. By modulating the activity of these receptors, CBD offers various therapeutic benefits including neuro protective, antiepileptic, anxiolytic, antipsychotic, and analgesic effects. In addition, CBD also exhibits anti-inflammatory and anti-cancer properties [17-18].

CBD has been extensively investigated in various types of cancer cells, including glioblastoma, skin, prostate, pancreas, colorectal and breast cancer [19]. Studies have consistently demonstrated the antproliferative properties of CBD, were achieved by the modulation of signaling pathways and the tumor microenvironment as a result of binding to CB1 or CB2 receptors, induction of apoptosis and cell cycle arrest and the inhibition of cell adhesion in cancer cells [18-20]. The primary objective of this meta-analysis is to determine whether CBD exhibits ant proliferative effects in triple-negative or positive receptor subtypes of breast cancer.

Methods

Search strategy

We conducted a comprehensive search of electronic databases, including PubMed, Scopus, Web of Science, EBSCO, ProQuest central, and ScienceDirect, to identify studies in English and Spanish that investigated the anti-tumor effects of cannabis derivatives on various breast cancer cell lines. To ensure a systematic identification of search terms, we used MeSH terms related to (("cell line"[MeSH Terms] OR ("cell"[All Fields] AND "line"[All Fields]) OR "cell line"[All Fields]) AND ("neoplasm s"[All Fields] OR

"neoplasms" [MeSH Terms] OR "neoplasms"[All Fields] OR "neoplasm"[All Fields]) AND ("cannabinoids"[MeSH Terms] OR "cannabinoids "[All Fields] OR "cannabinoid" [All Fields])), with different combinations to identify relevant records from all databases up to December 2022 (Supplementary material S1). The study was designed and conducted following the PRISMA guidelines.

Eligibility criteria and data extraction

The systematic review and meta-analysis included studies that met the eligibility criteria based on three factors: 1) in vitro preclinical trials, 2) inclusion of different breast cancer cell lines (triple negative and receptor positive), and 3) use of various cannabis derivatives such as CBD, Δ9-THC, cannabinol (CBN), cannabigerol (CBG). Studies that were excluded met one or more of the following exclusion criterias: duplication, insufficient data, publication before 2010, publication in a language other than English or Spanish, clinical trials, case reports, editor letters, reviews, abstracts, and comments. To ensure accuracy, automatic de-duplication and blinded screening by two independent reviewers (DT/LL) were performed and any discrepancies were resolved by discussion and consensus. Relevant studies were retrieved from various databases and stored in a reference management system. Data was extracted using a pre-established form that included key information such as title, publication year, first author name, cannabinoid names, the concentration of cannabinoids, breast cancer cell line type, techniques and assays used, effect on cell viability, migration, cell cycle, apoptosis and other relevant details.

Quality assessment

To evaluate the reliability and credibility of the methods employed in each study, we utilized the Cell Culture Study Quality Evaluation Guide (CCSQEG). This guide considers various factors, including the study design, methodologies, results, reports, validation, and potential bias or confounding factors. We established a minimum score for inclusion. Studies achieving a score of 6 or higher were considered as low risk for bias. Any discrepancies in the evaluation were resolved through consensus between the two authors (DT-LL).

Data Analysis

This systematic review and meta-analysis aimed to investigate the impact of different cannabidiol compounds on various cellular activities involved in the progression of breast cancer in vitro, including cell viability, proliferation, cell cycle, apoptosis, and migration. The effects of each compound on these cellular processes were assessed individually in different breast cancer cell lines. Open Meta[analysis] software was used to evaluate the effect of various cannabinoid derivatives on cell viability and graphical representations of the results were generated. The DerSimonian and Laird method was utilized to calculate the weighted average of the logarithms of odds ratios and a 95% confidence interval (CI) was set for all outcomes, including cell viability, cell cycle arrest stage, apoptosis, and other relevant characteristics.

Random effect models or fixed effect models were applied to estimate the odds ratios. Cochran's Q test and the I2 statistic were used to evaluate the heterogeneity of each pooled estimate. The significance level was defined as $p < 0.05$. The I2 test was used to assess heterogeneity across studies. An I2 value of 50% or less indicates homogeneity and a fixed-effects model is used, while an I2 value greater than 50%

indicates significant heterogeneity and a random-effects model is applied, funnel plot was using to determinate the risk of bias. We conducted a subgroup analysis to explore potential sources of substantial heterogeneity.

Relation maps between articles

To visualize the interrelationships among the articles included in our systematic review and meta-analysis, we utilized two software tools, Litmaps and Semantic Scholar. The software generated connecting lines between related articles and grouped them into distinct clusters based on their content. To ensure the accuracy of the visualizations, we carefully reviewed and analyzed the connections and clusters generated by the software and compared them with other visualizations and our interpretation of the data.

Results

Literature search

The PRISMA diagram in Figure 1 illustrates the literature search and screening process. The search strategy outlined in Supplementary material S1 was utilized to explore multiple databases, including PubMed, EBSCO, ProQuest Central, Scopus, Science Direct, and Web of Science. A total of 2585 articles were initially retrieved using the specified search terms. During the first screening phase, several articles were excluded. Specifically, 1532 duplicates, 117 articles published prior to 2010, and 1 article in a language other than English were removed. As a result, 935 articles were eligible and underwent further evaluation through title and abstract review.
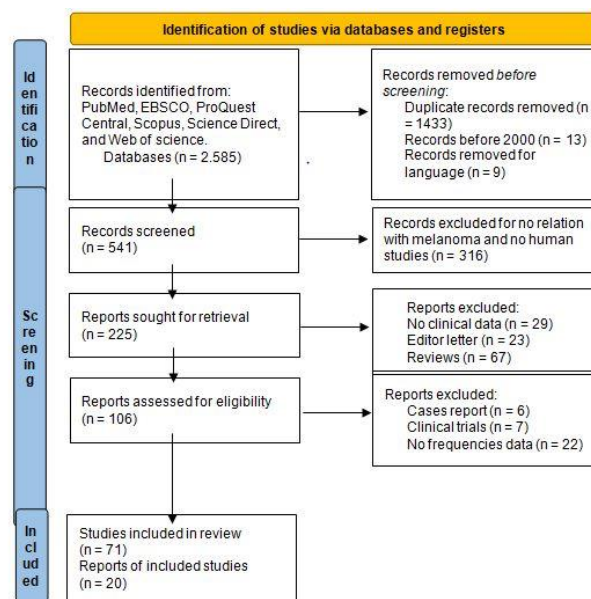

Identification of studies via databases and registers

Records identified from: PubMed, EBSCO, ProQuest Central, Scopus, Science Direct, and Web of science. Databases (n = 2.585)

Records removed *before screening*: Duplicate records removed (n = 1433) Records before 2000 (n = 13) Records removed for language (n = 9)

Records screened (n = 541)

Records excluded for no relation with melanoma and no human studies (n = 316)

Reports sought for retrieval (n = 225)

Reports excluded: No clinical data (n = 29) Editor letter (n = 23) Reviews (n = 67)

Reports assessed for eligibility (n = 106)

Reports excluded: Cases report (n = 6) Clinical trials (n = 7) No frequencies data (n = 22)

Studies included in review (n = 71) Reports of included studies (n = 20)

Figure 1: The schematic of PRISMA used in the meta-analysis.

Among these remaining articles, 786 records were deemed irrelevant based on the exclusion criteria applied. Consequently, 149 articles remained for a comprehensive review, and out of these, 19 studies

focusing on CBD in breast cancer cells were included in the qualitative synthesis. Litmaps and Semantic Scholar software were used to show the relationship between the 19 articles by generating connecting lines which in turn cluster the articles based on their content (Figure 2). The study had two main outcomes of interest. Firstly, it aimed to analyze the effect of various cannabinoid compounds on the cell viability of breast cancer cell lines in vitro. Secondly, it aimed to describe the effects of cannabinoid compounds on both triple-negative and receptor-positive breast cancer cells.
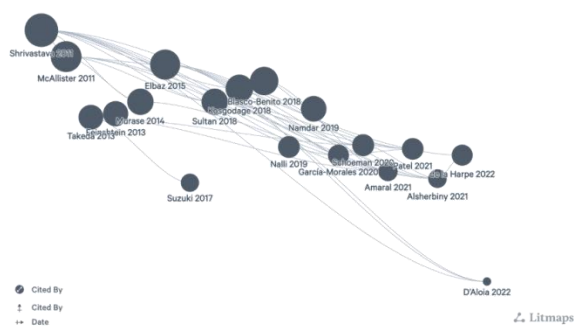

Figure 2: Relationship between the analyzed articles in the literature review on breast cancer cell lines and cannabinoid compounds using Litmaps.

Study Characteristics

The study focused on analyzing 19 selected articles, which provided valuable quantitative data on cell viability in various types of breast cancer cell lines (Table 1).

Title	Author	Year	Compounds	Concentration μM	Cell lines	Reference
Analyzing the role of cannabinoids as modulators of Wnt/β-catenin signaling pathway for their use in the management of neuropathic pain	Nalli Y	2019	Extract	1 - 100	MCF7	[21]
Appraising the "entourage effect": Antitumor action of a pure cannabinoid versus a botanical drug preparation in preclinical models of breast cancer	Blasco-Benito S	2018	THC	0 - 10	MCF7 T47D BT474 HCC1954 MDA-	[14]

					MD-231	
					SUM 159	
Cannabidiol (CBD) Is a Novel Inhibitor for Exosome and Microvesicle (EMV) Release in Cancer	Kosgodage U	2018	CBD	0 - 5	MDA-MD-231	[22]
Cannabidiol Antiproliferative Effect in Triple-Negative Breast Cancer MDA-MB-231 Cells Is Modulated by Its Physical State and by IGF-1	Alessia D	2022	CBD	0 - 50	MDA-MD-231	[23]
Cannabidiol enhances xenobiotic permeability through the human placental barrier by direct inhibition of breast cancer resistance protein: an ex vivo study	Feinshtein V.	2013	CBD	0 - 100	MCF7	[24]
Cannabidiol Induces Programmed Cell Death in Breast Cancer Cells by Coordinating the Cross-talk between Apoptosis and Autophagy	Shrivastava A	2011	CBD	0 - 10	MDA-MD-231 MCF7 SK-BR-3 ZR-75-1 MCF10A	[25]
Cannabidiol loaded extracellular vesicles sensitize triple-negative breast cancer to doxorubicin in both in-vitro and in vivo models	Patel N	2021	CBD	0 - 10	MDA-MD-231	[26]

Title	Author	Year	Compound	Concentration	Cell line	Ref
Cannabidiolic Acid-Mediated Interference with AP-1 Transcriptional Activity in MDA-MB-231 Breast Cancer Cells	Suzuki M	2017	CBDA	0 - 100	MDA-MD-231	[27]
Cannabinoid Combination Induces Cytoplasmic Vacuolation in MCF-7 Breast Cancer Cells	Schoeman R	2020	THC, CBG, CBN, and CBD	0 - 64	MDA-MD-231	[28]
					MCF7	
CBD activation of TRPV1 induces oxidative signaling and subsequent ER stress in breast cancer cell lines	Harpe A	2021	CBD	0 - 20	MCF7	[29]
					MDA-MD-231	
					MCF10A	
CBD Reverts the Mesenchymal Invasive Phenotype of Breast Cancer Cells Induced by the Inflammatory Cytokine IL-1β	Garcia L	2020	CBD	0 - 25	MCF7	[30]
					6D	
Modulation of the tumor microenvironment and inhibition of EGF/EGFR pathway: Novel anti-tumor mechanisms of Cannabidiol in breast cancer	Elbaz M	2015	CBD	0 - 15	SUM 159	[31]
					MDA-MD-231	
Novel mechanism of cannabidiol-induced apoptosis in breast cancer cell lines	Sultan A	2018	CBD	0 - 7	MDA-MD-231	[32]
					T47D	
Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation,	McAllister S	2011	CBD	0 - 10	MDA-MD-231	[33]

invasion, and metastasis						
Synergistic Interactions of Cannabidiol with Chemotherapeutic Drugs in MCF7 Cells: Mode of Interaction and Proteomics Analysis of Mechanisms	Alsherbiny M	2021	CBD	0 - 100	MCF7	[34]
Targeting multiple cannabinoid anti-tumour pathways with a resorcinol derivative leads to inhibition of advanced stages of breast cancer	Murase R	2014	THC and CBD	0 - 4	MDA-MD-231	[35]
Terpenoids and Phytocannabinoids Co-Produced in Cannabis Sativa Strains Show Specific Interaction for Cell Cytotoxic Activity	Namdar D	2019	THC and CBD	0 - 40	MDA-MD-231	[36]
Unveiling the mechanism of action behind the anti-cancer properties of cannabinoids in ER+ breast cancer cells: Impact on aromatase and steroid receptors	Amaral C	2021	CBD	0 - 20	MCF7	[37]
Δ9-Tetrahydrocannabinol Disrupts Estrogen-Signaling through Up-Regulation of Estrogen Receptor β(ERβ)	Takeda S	2013	THC	0 - 50	MCF7	[38]

Table 1*: Summary of the studies included in the systematic review and meta-analysis.

These studies were conducted in different countries around the world and were published between 2010 and December 2022. To ensure the reliability of the analysis, a rigorous bias assessment was performed using the CCSQEG method. As a result, six articles were excluded from the analysis due to

identified biases (Supplementary material S2). Additionally, three articles did not provide complete data on CBD for all the analyses conducted, and thus they were not included in the final evaluation.

A total of 10 articles were thoroughly analyzed for all the required assessments. The experimental procedure aimed to evaluate the effects of various cannabinoid-derived compounds on breast cancer cell lines, including both those with positive hormone receptors and triple-negative cell lines. Cell densities ranging from 2.5x 10^3 to 10 x 10^3 were utilized, and different concentrations of the compounds were introduced to the culture media. The standard compounds were tested within the concentration range of 0-200 µM, while extracts were tested within the range of 0-500 µM. After an incubation period of 24 to 72 hours, the cell viability was measured.

To determine viability, cellular metabolic activity methods such as MTT, XTT, MTS, and Reassuring were employed. Furthermore, the impact of different cannabinoid compounds on apoptosis was assessed using Annexin/PI and apoptosis detection tests. Cell migration and invasion were analysed using the Boyden chamber technique, and the cell cycle was studied through the propidium iodide (PI) method. However, the collected data from these assessments were deemed insufficient to be included in this systematic analysis.

Cell lines used in the studies

In the context of the analyzed 10 articles, a total of 10 distinct cell lines were investigated, encompassing both triple-negative and positive receptor cell lines. Among the triple-negative breast cancer cell lines, three were investigated: SUM 159, an invasive and metastatic line; MDA-MD-231, a highly invasive line; and 6D, a clone selected from non-invasive MCF-7 cells [21-22]. Additionally, the cell line SK-BR-3, characterized by HER2 expression, and several cell lines with positive hormone receptors and non-metastatic properties including MCF7, T47D, and ZR-75-1 were also examined [21-23]. Notably, one article also involved the use of a non-tumoral breast cell line, MCF10A, for comparison with tumoral breast cancer cell lines [25].

The analyzed cannabinoids

Cannabinoids, are categorized into three maingroups: endocannabinoids, synthetic cannabinoids, and phytocannabinoids derived from plants, were the focus of analysis in this study [25]. The data examined in the article revealed a diverse range of plant cannabinoid extracts and pure compounds, showcasing heterogeneity among them. Notably, the studied cannabinoids included Δ⁹-THC, CBG, CBN, and CBD. Among these compounds, CBD emerged as the most extensively studied in terms of its effects on breast cancer cell lines, with sufficient data available to describe its impact.

Determination of the effect of cannabinoids on breast cancer cell viability

To determine the activity of different cannabinoids on breast cancer cell lines, data extraction was performed from all relevant articles. Several parameters were considered which include the number of cells used, the concentration of compounds, and the 50% inhibitory concentration (IC50) for each cell line and compound. The extensively studied compounds included THC, CBD, and total extracts. To analyse the association between the effects of cannabinoids and cell viability, averages were calculated

for each compound and subgroup of cell lines which include total cell lines, cell lines with positive receptors, and triple-negative cell lines. The obtained data was used to determine the effect of cannabinoids on breast cancer cells and their impact on cell viability. The average IC50 for all analyzed cells was 9.0 µM for CBD and 12.2 µM for THC. For cell lines with positive receptors, the average IC50 was 10.8 µM for CBD and 14.5 µM for THC, while for triple-negative cells, the average IC50 was 7.5 µM for CBD and 9.5 µM for THC.

From the comparison of results between pure compounds and extracts, it was identified that higher concentrations of extracts were required to achieve similar outcomes compared to CBD and THC compounds. In all three analyzed groups, THC exhibited greater variability in inhibiting cell viability and proliferation of tumor cells than CBD. In addition, the data did not allow for a determination of the effect of THC on cell viability. As a preliminary finding, CBD inhibited the proliferation of breast cancer tumor cells, regardless of their classification.

The results of the meta-analysis revealed significant findings regarding the protective effect of CBD against tumour growth in breast cancer cell lines. The odds ratio (OR) was calculated to be 0.531 with a 95% confidence interval (CI) from 0.429 to 0.656, and a p-value less than 0.001 (Figure 3). These results indicate a strong association between CBD administration and the inhibition of tumour growth in breast cancer cells.
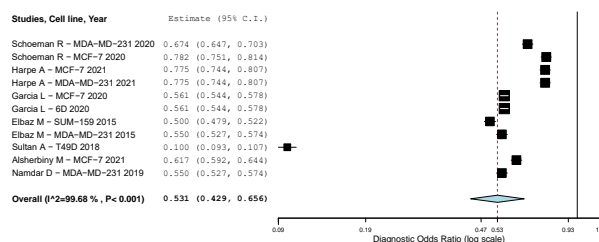

Figure 3: The forest plot shows the CBD influence on the viability of triple-negative and receptor-positive breast cancer cells.

Furthermore, the positive effect of CBD was observed across different types of breast cancer cell lines. In cell lines with positive receptors, the odds ratio was 0.579 with a CI 95% of 0.476 to 0.704, and a p-value less than 0.001 (Figure 4). This suggests that CBD effectively suppresses tumor growth in breast cancer cells expressing positive receptors.
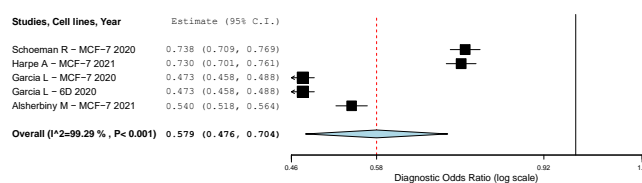
Figure 4: The forest plot of the results of the CBD effect on the viability of breast cancer cells with positive receptors.

Similarly, in triple-negative cell lines, the odds ratio was 0.670, with a 95% CI of 0.594 to 0.755, and a p-value less than 0.001 (Figure 5). This signifies that CBD exhibits its protective effect against tumor growth even in the absence of hormone receptors, which is a characteristic feature of triple-negative breast cancer. The data from this meta-analysis provide strong evidence supporting the use of CBD for inhibiting tumor growth and promoting cell viability in breast cancer cell lines, irrespective of receptor status.
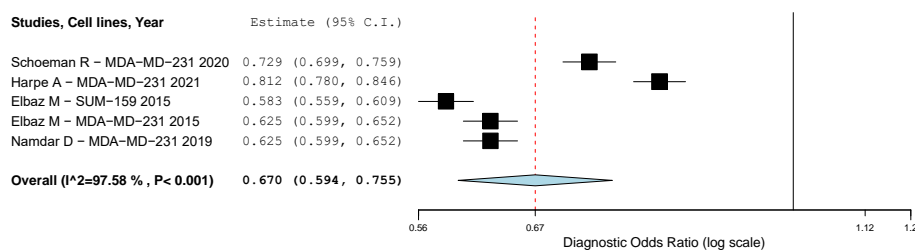
Figure 5: Results of the CBD effect on the viability of triple-negative breast cancer cells.

The standard error-based funnel plot show Figure 6 shows no significant asymmetry making it unlikely that the observed association was caused by publication bias.
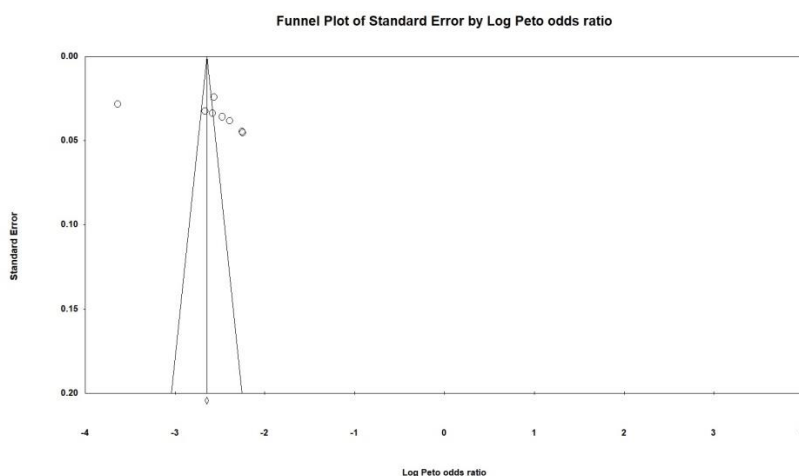
Figure 6: Standard error-based funnel plot of the result of relationship between CBD and all breast cancer cell lines.

Effect of cannabinoids on cellular division

In the context of investigating the effects of various cannabinoid derivatives on breast cancer cell lines, a comprehensive set of experiments were conducted to elucidate their impact on cell cycle progression. To achieve this, a combination of propidium iodide (PI) staining and flow cytometry analysis was employed, enabling the determination of the distribution of cells across different cell cycle phases, namely G0/G1, S, and G2/M.

The results obtained from these experiments revealed an intriguing finding regarding the potential of CBD to induce cell cycle arrest in breast cancer cells. Specifically, CBD demonstrated the remarkable ability to specifically arrest the cell cycle at the G1 phase when administered at concentrations below 2.5 µM in MDA-MD-231 cells, in comparison to the control group [26]. Furthermore, similar outcomes were observed in the MCF7 cell line, another well-established breast cancer model. In this case, both CBD and another prominent cannabinoid, THC, exhibited a significant arrest in the G0/G1 phase when administered at concentrations of 5 µM, as compared to the negative control [27]. These experimental findings provide valuable insights into the specific effects of CBD and THC on the cell cycle distribution of breast cancer cells.

Effect of cannabinoids on the induction of apoptosis

Various techniques were employed to investigate the effects of cannabinoids on apoptosis, reactive oxygen species (ROS) production, mitochondrial membrane potential loss, and caspase 9 or 7 activity in MCF7 cells. The methods used included the Cell Death Detection ELISA PLUS kit, Annexin V-CF Blue, 7-Aminoactinomycin D (7-AAD), Western Blot, and others. Both CBD and THC were found to enhance ROS production. Additionally, the activation of caspases 7 and 9 was observed within two days at concentrations below 10 µM [27]. Notably, CBD treatment resulted in greater cell death in the MDA-MD-231 cell line compared to the MCF7 and MCF10A cell lines. This phenomenon was attributed to the higher levels of ROS induced by CBD, distinguishing it from other compounds like THC, CBG, and CBN [24].

Cannabinoids` effect on breast cancer cell line ability to migrate and invade

Migration and invasion processes play a crucial role in modulating the epithelial-mesenchymal transition and cancer progression. The effects of different cannabinoid compounds were investigated for their effect on the migratory and invasive capacity of breast cancer cell lines using the Boyden chamber and the wound healing assay. In a wound healing assay, treatment with CBD at 6 µM diminished the migratory capacity of MDA-MD-231 and SUM159 cells and reduced the expression of MMP2 and MMP9 [31]. Furthermore, treatment of MDA-MB-231 cells with CBD significantly reduced the expression of proteins such as Integrin α-5, Twist, Glypican-1, Glypican-6, and Smad-2 compared to the negative control [26]. CBD at concentrations below 10 µM induced a reduction in the number of Ki-67 positive metastatic MDA-MB23 cells (a marker of cellular proliferation) [28] and inhibited the wound closure of the 6D cell line (MCF-7 with malignant phenotype) [22].

Discussion

Breast cancer is a major health concern that affects millions of women globally. Tumour cells stop responding to existing treatments and become chemoresistant. Therefore, the development of novel treatment strategies is essential to provide treatment alternatives and improve the survival rate [3, 30]. Cannabinoids are one of the options that have been investigated for their effects on different breast tumours [27, 30]. The limited studies available on the effect of cannabinoid treatment on breast cancer show that concentrations above 15 µM of CBD and THC can reduce the viability of positive receptor cells lines (MCF-7, T47D, and ZR-75-1) between 30 - 40% and up to 50% for triple-negative cell lines (SUM159 and MDA-MD-231) [22, 24, 31-33].

Studies have demonstrated that both THC and CBD have anticancer activities in different tumor cell lines. For instance, treatment of head and neck squamous cell carcinoma (HNSCC) and the human brain cancer cell line U87MG with low concentrations of CBD and CBD and THC combination caused a significant reduction in cell viability in a time-dependent manner [34-35]. Lung cancer cell lines, A549 and H1299 treated with CBD for 72 hours showed a cytotoxic effect [36]. In leukemias, CBD and THC treatments cause cell cycle arrest, by increasing the percentage of cells in the G0/G1 phase via several mechanisms. For instance, in chronic myeloid leukemia, it has been reported that CBD inhibited the transient receptor potential vanload type-2 (TRPV2), and in multiple myeloma, THC induced cell cycle arrest in the G1 phase after 24 hours post-treatment [37-39]. Similarly, in cholangiocarcinoma and gastric cancer, CBD induced cell cycle arrest at G0/G1and decreased the number of cells in the S phase by inducing DNA damage, interacting with TRPV channels and inhibiting Cyclin-dependent kinase 2 or cyclin E [40-42]. In the triple negative breast cancer cell line MDA-MD-231, CBD induced cell cycle arrest as shown by an increase of cells in the G1 and sub-G0 phases and a decrease in the number of cells in the S phase [26, 31, 43, and 44].

According to the available data, treatment with CBD in a dose-dependent manner induce apopt osis and autophagy by up regulating the expression of Caspase-3, -7, and -9 in HNSCC (SCC15, Hep2, and FaDu) [34, 45, 46]. Treatment of acute lymphoblastic leukemia cells with CBD caused an increase in ROS, LC3-II, and calcium metabolism [45]. Similarly, in estrogen receptor-positive and negative breast cancer cells treated with CBD and the combination of CBD with other cannabinoids or chemotherapeutic drugs also

induce autophagy and apoptosis in a concentration-dependent manner by inducing endoplasmic reticulum stress signified by the increase in LC3-I, LC3-II, pro-PARP, cleaved PARP and cleavage of procaspases -3, -7, and -9 [31, 47, 48].

Metastatic disease has a high mortality rate [49]. Cannabinoids have shown anti-invasion and anti-migration effects. Several research groups mainly conducted in vitro assays with glioblastoma, adenocarcinoma, HNSCC, and mesothelioma cells to test the effect of CBD at various concentrations [34, 49-51]. From the limited available studies, treatment of MDA-MB-23 with CBD caused a reduction in cell migration by inhibiting cAMP-dependent protein kinase A, ERK, and AKT signalling, and modulating the tumour microenvironment [28, 32, 52]. In addition, it was shown through a colony formation assay that treatment with CBD for 24 hours reduced the ability of chronic myeloid leukemia cell lines to form colonies, reduce the size of the colonies of cholangiocarcinoma cell lines (HuCC-T1 and Mz-ChA-1) and induce a cytotoxic effect in HNSCC [34, 37, 40]. Collectively, recent studies show that CBD has anti-migratory effects across different types of cancer cell lines including breast cancer, hence making CBD a potential therapeutic option. One of the major limitations in our meta-analysis is that few studies investigated the anti-cancer effect of cannabinoids on breast cancer cell lines. Furthermore, these studies used similar approaches to explore the anti-cancer effect of cannabinoids. Not all breast cancer cell lines were evaluated, and most of the studies tested the effect of CBD, with only a few studies testing the effect of THC, CBN, and CBG.

Conclusion

Our systematic review and meta-analysis identified that CBD and THC show anti-cancer effects on several cancer cell lines by inhibiting cellular proliferation, migration and invasion and promoted cell death by inducing apoptosis and autophagy. Collectively, studies show that CBD and THC may have potential to be used as a treatment individually or in combination with other chemotherapeutic agents. Although the most tested cannabinoids on breast cancer cell lines are CBD and THC, it is worthwhile to investigate the anti-cancer effect of other cannabinoids such as cannabigerovarin, cannabigerolic acid and cannabichromevarin. Finally, more studies are required to identify the mechanisms by which cannabinoids exert their anti-cancer effects.

Declaration

Authors' contributions

D.T, data collection or analysis and interpretation of data; statistical analysis; writing of the manuscript or critical review of important intellectual content; critical review of the literature, administrative, technical, and material support. L.L.S, the study concept and design; analysis and interpretation of data; statistical analysis; writing of the manuscript or critical review of important intellectual content; data

References

1. https://www.cancer.org/cancer/melanoma-skin-cancer/about/key-statistics.html.
2. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, et al. (2022) Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 66:15-23
3. https://www.who.int/news-room/fact-sheets/detail/breast-cancer.
4. 4.http://globocan.iarc.fr/old/burden.asp?selection_pop=224900&Textp=World&selection_cancer=16120&Text-c=Melanoma+of+skin&pYear=8&type=1&window=1&submit=%20Execute.
5. Liu C, Mohan SC, Wei J, Seki E, Liu M, et al. (2022) Breast cancer liver metastasis: Pathogenesis and clinical implications. Front Oncol. 12:1043771.
6. Klein CA. (2020) Cancer progression and the invisible phase of metastatic colonization. Nat Rev Cancer. 20(11):681-94.
7. Seyfried TN, Huysentruyt LC. (2013) On the origin of cancer metastasis. Crit Rev Oncog. 18(1-2):43-73.
8. Suhail Y, Cain MP, Vanaja K, Kurywchak PA, Levchenko A, et al. (2019) Systems Biology of Cancer Metastasis. Cell Syst. 9(2):109-27.
9. Rajaram P, Chandra P, Ticku S, Pallavi B, Rudresh K, et al. (2017) Epidermal growth factor receptor: Role in human cancer. Indian J Dent Res. 28(6): 687-94.
10. Wang Z. (2017) ErbB Receptors and Cancer. Methods Mol Biol. 1652:3-35.
11. Kumar P, Aggarwal R. (2016) An overview of triple-negative breast cancer. Arch Gynecol Obstet. 293(2):247-69.
12. Jin X, Mu P. (2015) Targeting Breast Cancer Metastasis. Breast Cancer (Auckl). 9(Suppl 1):23-34.
13. Smith RA, Saslow D, Sawyer KA, Burke W, Costanza ME, et al. (2003) American Cancer Society guidelines for breast cancer screening: update 2003. CA Cancer J Clin. 53(3):141-69.
14. Blasco-Benito S, Seijo-Vila M, Caro-Villalobos M, Tundidor I, Andradas C, et al. (2018) Appraising the "entourage effect": Antitumor action of a pure cannabinoid versus a botanical drug preparation in preclinical models of breast cancer. Biochem Pharmacol. 157:285-93.
15. Liu J, Gan M, Lin Z, Deng Q, Deng J, et al. (2022) Clinical Features and Prognosis Analysis of Hormone Receptor-Positive, HER2-Negative Breast Cancer with Differential Expression Levels of Estrogen and Progesterone Receptors: A 10-Year Retrospective Study. Breast J. 2022:5469163.
16. Kisková T, Mungenast F, Suváková M, Jäger W, Thalhammer T. (2019) Future Aspects for Cannabinoids in Breast Cancer Therapy. Int J Mol Sci. 20(7):1673.
17. Peng J, Fan M, An C, Ni F, Huang W, et al. (2022) A narrative review of molecular mechanism and therapeutic effect of cannabidiol (CBD). Basic Clin Pharmacol Toxicol. 130(4):439-56.
18. Castillo J, Canseco A, Cutler Te, and Francisco L. (2023) The Polypharmacological Effects of Cannabidiol. Molecules. 28(7):3271.

19.   Mangal N, Erridge S, Habib N, Sadanandam A, Reebye V, et al. (2021) Cannabinoids in the landscape of cancer. J Cancer Res Clin Oncol. 147(9):2507-34.

20.   Pagano C, Navarra G, Coppola L, Avilia G, Bifulco M, et al. (2022) Cannabinoids: Therapeutic Use in Clinical Practice. Int J Mol Sci. 23(6):3344.

21.   Rizwan A, Cheng M, Bhujwalla ZM, Krishnamachary B, et al. (2015) Breast cancer cell adhesome and degradome interact to drive metastasis. NPJ Breast Cancer. 1:15017.

22.   García-Morales L, Castillo AM, Tapia Ramírez J, Zamudio-Meza H, Domínguez-Robles MDC, et al. (2020) CBD Reverts the Mesenchymal Invasive Phenotype of Breast Cancer Cells Induced by the Inflammatory Cytokine IL-1β. Int J Mol Sci. 21(7):2429.

23.   Karcini A, Lazar IM. (2022) The SKBR3 cell-membrane proteome reveals telltales of aberrant cancer cell proliferation and targets for precision medicine applications. Sci Rep. 12(1):10847.

24.   de la Harpe A, Beukes N, Frost CL. (2022) CBD activation of TRPV1 induces oxidative signaling and subsequent ER stress in breast cancer cell lines. Biotechnol Appl Biochem. 69(2):420-30.

25.   Śledziński P, Nowak-Terpiłowska A, Zeyland J. (2020) Cannabinoids in Medicine: Cancer, Immunity, and Microbial Diseases. Int J Mol Sci. 22(1):263.

26.   Patel N, Kommineni N, Surapaneni SK, Kalvala A, Yaun X, et al. (2021) Cannabidiol loaded extracellular vesicles sensitize triple-negative breast cancer to doxorubicin in both in-vitro and in vivo models. Int J Pharm. 607:120943.

27.   Amaral C, Trouille FM, Almeida CF, Correia-da-Silva G, Teixeira N. (2021) Unveiling the mechanism of action behind the anti-cancer properties of cannabinoids in ER+ breast cancer cells: Impact on aromatase and steroid receptors. J Steroid Biochem Mol Biol. 210:105876.

28.   Murase R, Kawamura R, Singer E, Pakdel A, Sarma P, et al. (2014) Targeting multiple cannabinoid anti-tumour pathways with a resorcinol derivative leads to inhibition of advanced stages of breast cancer. Br J Pharmacol. 171(19):4464-77.

29.   Tomko A, O'Leary L, Trask H, Achenbach JC, Hall SR, et al. (2019) Antitumor Activity of Abnormal Cannabidiol and Its Analog O-1602 in Taxol-Resistant Preclinical Models of Breast Cancer. Front Pharmacol. 10:1124.

30.   Baram L, Peled E, Berman P, Yellin B, Besser E, et al. (2019) The heterogeneity and complexity of Cannabis extracts as antitumorales agents. Oncotarget. 10(41):4091-106.

31.   Schoeman R, Beukes N, Frost C. (2020) Cannabinoid combination induces cytoplasmic vacuolation in MCF-7 breast cancer cells. Molecules. 25(20):4682.

32.   Elbaz M, Nasser MW, Ravi J, Wani NA, Ahirwar DK, et al. (2015) Modulation of the tumor microenvironment and inhibition of EGF/EGFR pathway: Novel anti-tumor mechanisms of Cannabidiol in breast cancer. Mol Oncol. 9(4):906-19.

33.   Sultan AS, Marie MA, Sheweita SA. (2018) Novel mechanism of cannabidiol-induced apoptosis in breast cancer cell lines. Breast. 41:34-41.

34.   Go YY, Kim SR, Kim DY, Chae SW, Song JJ. (2020) Cannabidiol enhances cytotoxicity of anti-cancer drugs in human head and neck squamous cell carcinoma. Sci Rep. 2020;10(1):20622.

35.   López-Valero I, Saiz-Ladera C, Torres S, Hernández-Tiedra S, García-Taboada E, et al. (2018) Targeting Glioma Initiating Cells with A combined therapy of cannabinoids and temozolomide. Biochem Pharmacol. 157:266-74.

36.   Todorova J, Lazarov LI, Petrova M, Tzintzarov A, Ugrinova I. (2021) The antitumor activity of cannabidiol on lung cancer cell lines A549 and H1299: the role of apoptosis. Biotechnology & Biotechnological Equipment. 35(1):873-9.

37.   Maggi F, Morelli MB, Tomassoni D, Marinelli O, Aguzzi C, et al. (2022) The effects of cannabidiol via TRPV2 channel in chronic myeloid leukemia cells and its combination with imatinib. Cancer Sci. 113(4):1235-49.

38.   Nabissi M, Morelli MB, Offidani M, Amantini C, Gentili S, et al. (2016) Cannabinoids synergize with carfilzomib, reducing multiple myeloma cells viability and migration. Oncotarget. 7(47):77543-57.

39.   Scott KA, Dalgleish AG, Liu WM. (2017) Anticancer effects of phytocannabinoids used with chemotherapy in leukaemia cells can be improved by altering the sequence of their administration. Int J Oncol. 51(1):369-77.

40.   Viereckl MJ, Krutsinger K, Apawu A, Gu J, Cardona B, et al. (2022) Cannabidiol and Cannabigerol Inhibit Cholangiocarcinoma Growth In Vitro via Divergent Cell Death Pathways. Biomolecules. 12(6):854.

41.   Zhang X, Qin Y, Pan Z, Li M, Liu X, et al. (2019) Cannabidiol Induces Cell Cycle Arrest and Cell Apoptosis in Human Gastric Cancer SGC-7901 Cells. Biomolecules. 2019;9(8):302.

42.   Zhong N, Li D, Wang B, Kovalchuk O, Kovalchuk I. (2023) Cannabinol inhibits cell growth and triggers cell cycle arrest and apoptosis in cancer cells. Biocatalysis and Agricultural Biotechnology. 48:102627.

43.   D'Aloia A, Ceriani M, Tisi R, Stucchi S, Sacco E, et al. (2022) Cannabidiol Antiproliferative Effect in Triple-Negative Breast Cancer MDA-MB-231 Cells Is Modulated by Its Physical State and by IGF-1. Int J Mol Sci. 23(13):7145.

44.   McAllister SD, Murase R, Christian RT, Lau D, Zielinski AJ, et al. (2011) Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation, invasion, and metastasis. Breast Cancer Res Treat. 129(1):37-47.

45.   Olivas-Aguirre M, Torres-López L, Valle-Reyes JS, Hernández-Cruz A, Pottosin I, et al. (2019) Cannabidiol directly targets mitochondria and disturbs calcium homeostasis in acute lymphoblastic leukemia. Cell Death Dis. 10(10):779.

46.   Armstrong JL, Hill DS, McKee CS, Hernandez-Tiedra S, Lorente M, et al. (2015) Exploiting cannabinoid-induced cytotoxic autophagy to drive melanoma cell death. J Invest Dermatol. 135(6):1629-37.

47.   Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A. (2011) Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and AutophagyCBD induces programmed cell death in breast cancer cells. Mol Cancer Ther. 10(7):1161-72.

48.   Alsherbiny MA, Bhuyan DJ, Low MN, Chang D, Li CG. (2021) Synergistic interactions of cannabidiol with chemotherapeutic drugs in mcf7 cells: Mode of interaction and proteomics analysis of mechanisms. Int J Mol Sci. 22(18):10103.

49.   Colvin EK, Hudson AL, Anderson LL, Kumar RP, McGregor IS, et al. (2022) An Examination of the Anti-Cancer Properties of Plant Cannabinoids in Preclinical Models of Mesothelioma. Cancers (Basel). 14(15):3813.

50.   Lah TT, Novak M, Pena Almidon MA, Marinelli O, Žvar Baškovič B, et al. (2021) Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma. Cells. 10(2):340.

51.   Luongo M, Marinelli O, Zeppa L, Aguzzi C, Morelli MB, et al. (2020) Cannabidiol and Oxygen-Ozone Combination Induce Cytotoxicity in Human Pancreatic Ductal Adenocarcinoma Cell Lines. Cancers (Basel). 12(10):2774.

52.   Takeda S, Okajima S, Miyoshi H, Yoshida K, Okamoto Y, et al. (2012) Cannabidiolic acid, a major cannabinoid in fiber-type cannabis, is an inhibitor of MDA-MB-231 breast cancer cell migration. Toxicol Lett. 214(3):314-19.